Multiple functions for the poly(A)-binding protein in mRNA decapping and deadenylation in yeast

Giordano Caponigro and Roy Parker¹

Department of Molecular and Cellular Biology and Howard Hughes Medical Institute, University of Arizona, Tucson, Arizona 85721 USA

The first step in the decay of many eukaryotic mRNAs is shortening of the poly(A) tail. In yeast, deadenylation leads to mRNA decapping and subsequent 5' → 3' exonucleolytic degradation of the transcript body. We have determined that the major poly(A)-binding protein Pab1p plays at least two critical roles in this pathway. First, mRNAs in *pab1Δ* strains were decapped prior to deadenylation. This observation defines a new function for Pab1p as an inhibitor of mRNA decapping. Moreover, mutations that inhibit mRNA turnover suppress the inviability of a *pab1Δ* mutation, suggesting that premature mRNA decapping in *pab1Δ* strains contributes to cell death. Second, we find that Pab1p is not required for deadenylation, although in its absence poly(A) tail shortening rates are significantly reduced. In addition, in the absence of Pab1p, newly synthesized mRNAs had poly(A) tails longer than those in wild-type strains and showed an unexpected temporal delay prior to the initiation of deadenylation and degradation. These results define new and critical functions for Pab1p in the regulation of mRNA decapping and deadenylation, two important control points in the specification of mRNA half-lives. Moreover, these results suggest that Pab1p functions in additional phases of mRNA metabolism such as mRNP maturation.

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mRNA decay is an important control point in post-transcriptional gene regulation. Half-lives of different mRNAs can vary over a wide range, and there are a large number of examples in which decay rates of specific transcripts are altered in response to environmental cues (for review, see Belasco and Brawerman 1993). One major pathway of mRNA decay in eukaryotes is initiated by shortening of the poly(A) tail, which is followed by degradation of the transcript body (for review, see Bernstein and Ross 1989; Sachs 1993; Decker and Parker 1994). In yeast, following deadenylation, degradation of the body of the transcript can occur by removal of the 5' monomethyl cap (decapping) and subsequent 5' → 3' exonucleolytic digestion of the mRNA (Decker and Parker 1993; Hsu and Stevens 1993; Muhlrud et al. 1994, 1995). To date, there is no direct evidence for such a pathway in more complex eukaryotes; however, mRNAs lacking the cap structure are rapidly degraded in many eukaryotic cells (e.g., Drummond et al. 1985), and enzymatic activities that could catalyze the removal of the cap structure and subsequent 5' → 3' degradation of the transcript have been described in mammalian cells (e.g., Coutts

and Brawerman 1993). Thus, deadenylation-dependent decapping followed by 5' → 3' exonucleolytic decay may be a conserved eukaryotic mRNA decay mechanism.

Individual yeast mRNAs that degrade through the deadenylation-dependent decapping pathway can have very different half-lives as a consequence of mRNA-specific rates of deadenylation and decapping. For example, the relatively stable *PGK1* transcript ($t_{1/2} = 35'$) has a slow rate of deadenylation (4 adenylate residues per minute) and is also decapped slowly. In contrast, the unstable *MFA2* transcript ($t_{1/2} = 4.5'$) has a faster rate of deadenylation (13 adenylate residues per minute) and is decapped rapidly (Decker and Parker 1993; Muhlrud et al. 1994, 1995). Given these differences, to understand how mRNA decay rates are specified it is critical to determine the mechanisms that control deadenylation and decapping.

In both yeast and more complex eukaryotes, transcripts deadenylate at different rates due, in part, to specific sequence elements within mRNAs. One example of such a sequence is the UUAUUUAUU sequence motif found in many labile mammalian transcripts (Wilson and Triesman 1988; Shyu et al. 1991; Chen and Shyu 1994; Lagnado et al. 1994; Zubiaga et al. 1995). Factors that recognize some of these elements have been identi-

¹Corresponding author.

fied; however, it is not yet known how, or if, these factors act during deadenylation (Malter 1989; Bohjanen et al. 1991; Brewer 1991; Vakalopoulou et al. 1991; You et al. 1992; Katz et al. 1994). The only protein that has been found to influence deadenylation *in vivo* is the yeast poly(A)-binding protein, Pab1p. This conclusion is based on the observation that depletion of Pab1p results in an increase in the length of poly(A) tails in the total RNA population (Sachs and Davis 1989). This result has been interpreted to indicate that Pab1p is required for deadenylation, presumably for a Pab1p-dependent poly(A) nuclease to function (Lowell et al. 1992; Sachs and Dardorff 1992).

Similar to deadenylation, rates of decapping vary significantly between transcripts and are influenced by specific features of an mRNA. For instance, sequences within a transcript can significantly affect rates of mRNA decapping following deadenylation (Muhlrads and Parker 1992; Decker and Parker 1993; Muhlrads et al. 1995). In addition, because decapping normally occurs after deadenylation, the poly(A) tail itself is somehow involved in inhibiting the decapping reaction (Decker and Parker 1993; Muhlrads et al. 1994, 1995). The mechanism by which the poly(A) tail inhibits decapping is unknown, but this inhibition suggests that a functional interaction exists between the 5' and 3' ends of an mRNA. The existence of such an interaction is also suggested by the observations that the poly(A) tail and specific sequences within 3'-untranslated regions (3' UTRs) of mRNAs affect translation initiation (for review, see Jackson and Standart 1990; Curtis et al. 1995). Determining the nature of this interaction is likely to be critical for an understanding of both translation and mRNA turnover.

Pab1p may be a key factor involved in mediating the effects of a 5' → 3' interaction, such as the inhibition of mRNA decapping by the poly(A) tail. It is the major cytoplasmic poly(A)-binding protein and is highly conserved among eukaryotic organisms (Sachs et al. 1986; Grange et al. 1987; Zelus et al. 1989; Belostotsky and Meagher 1993). Additionally, several lines of evidence argue that Pab1p plays a role in stimulating translation initiation (Grossi de Sa et al. 1988; Sachs and Davis 1989), which suggests that this protein can influence events at the 5' end of an mRNA while bound to the 3' poly(A) tail.

We have investigated the roles of Pab1p in mRNA turnover and found three defects that result from the loss of Pab1p. First, mRNAs in *pab1Δ* strains were decapped prior to deadenylation, indicating that Pab1p is required for the normal coupling of poly(A) tail removal and decapping. Moreover, a *pab1Δ* mutation was suppressed by mutations in genes involved in mRNA decay, including the *XRN1* gene, which encodes a major 5' → 3' exonuclease (Larimer and Stevens 1990). This result suggests that aberrant mRNA decapping may contribute to lethality resulting from loss of Pab1p. Second, poly(A) shortening did not require Pab1p, although in its absence rates of deadenylation were significantly reduced. Third, in the absence of Pab1p, newly synthesized mRNAs had

poly(A) tails that were longer than their counterparts in wild-type strains and exhibited a temporal lag prior to deadenylation and decay. These data indicate that Pab1p functions in two important steps in a general pathway of mRNA decay in yeast. Furthermore, these data suggest that Pab1p may play a role, or roles, in additional phases of mRNA biogenesis.

Results

pab1Δ strains aberrantly decap mRNAs prior to deadenylation

We first examined the role of Pab1p in mediating inhibition of mRNA decapping by the poly(A) tail. To do so, a poly(G) tract, which forms a strong secondary structure (Zimmerman et al. 1975; Williamson et al. 1989), was inserted into the 3' UTRs of the unstable *MFA2* and stable *PGK1* mRNAs (termed *MFA2pG* and *PGK1pG*, respectively). The presence of this structure in these mRNAs results in the trapping of decay products that arise following decapping of these transcripts (Vreken and Raue' 1992; Decker and Parker 1993). These decay products are trimmed to the 5' side of the poly(G) insertion and normally have oligo(A)-length (0–15 nucleotides) poly(A) tails because mRNA decapping and 5' → 3' digestion occur subsequent to poly(A) shortening (Decker and Parker 1993; Muhlrads et al. 1994). If Pab1p is required for the poly(A) tail to inhibit decapping, then these decay products would be expected to have long poly(A) tails in the absence of Pab1p (shown schematically in Fig. 1A).

Because *PAB1* is an essential gene, these experiments were first performed in a *pab1Δ* strain that harbored a suppressor of the *pab1* deletion. The suppressor used was a disruption of the *SPB2* gene, which encodes the large ribosomal subunit protein RPL46 (Leer et al. 1985; Sachs and Davis 1989). We examined decay products arising from the *MFA2pG* and *PGK1pG* transcripts in both wild-type (yRP840, Table 1) and *spb2Δ* (yRP843) strains (Fig. 1, B and C, for *MFA2pG* and *PGK1pG*, respectively; see Materials and methods). In both strains these decay products had oligo(A)-length poly(A) tails as determined by comparison to identical samples lacking poly(A) tails because of treatment with RNase H and oligo(dT) [Fig. 1B,C, RNase H + oligo(dT) lanes]. This result was consistent with earlier observations (Decker and Parker 1993; Muhlrads et al. 1994) and indicates that the *spb2* lesion by itself does not alter the dependence of decapping on deadenylation. In contrast, in the *pab1Δ* *spb2Δ* strain (yRP881), decay products for both mRNAs had poly(A) tails up to 75 nucleotides in length, as determined by comparison to identical poly(A)-minus mRNA samples (Fig. 1B,C). The observation that these decay intermediates are generated with long poly(A) tails in *pab1Δ* mutants strongly suggests that the requirement for poly(A) tail removal prior to decapping has been bypassed in the absence of the *PAB1* gene product.

Figure 1. Decay products from the MFA2pG and PGK1pG transcripts have long poly(A) tails in *pab1Δ* yeast. (A) Pathways of mRNA decay. The branch of the diagram showing deadenylation-dependent decapping depicts an established pathway of mRNA decay in yeast. The initial phase of decay entails shortening of the poly(A) tail to an oligo(A) form, followed by mRNA decapping. Subsequent 5' → 3' digestion of the transcript results in the production of a decay product trimmed to the 5' side of the poly(G) insertion, which has an oligo(A)-length poly(A) tail. The branch of the pathway showing deadenylation-independent decapping depicts the production of decay products with long poly(A) tails that would result from decapping prior to poly(A) tail removal. (B,C) Steady-state mRNA samples from *spb2Δ*, wild-type, and *pab1Δ spb2Δ* yeast strains were resolved on 6% acrylamide/8 M urea Northern gels either following or without (as indicated) removal of poly(A) tails with RNase H and oligo(dT). (B) Poly(A) tail lengths on the MFA2pG transcript and decay product. The upper and lower bands in the RNase H and oligo(dT) lanes correspond to poly(A)-minus full-length MFA2pG mRNA and MFA2pG decay products respectively. The size heterogeneity of these same bands in the untreated samples is attributable to varying poly(A) tail lengths on these mRNAs. A schematic of the full-length mRNA and decay product, as well as poly(A) tail lengths on these mRNA species, is shown at right. Here, and in all subsequent figures, poly(A) tail lengths were determined by comparison of bands to size standards and the poly(A)-minus mRNA species generated by cleavage with RNase H and oligo(dT) (data not shown). (C) Poly(A) tail lengths on the PGK1pG decay product. The prominent band in the RNase H and oligo(dT)-treated samples corresponds to the poly(A)-minus PGK1pG decay product; size heterogeneity of these bands in the untreated samples is attributable to poly(A) tails of varying lengths on the decay products. A schematic of the decay product and poly(A) tail lengths is shown at right. Lower levels of MFA2pG and PGK1pG mRNA in the wild-type lanes relative to the *spb2Δ* lanes in B and C is attributable to unequal loading as judged by standardization to the *scr1* transcript as described previously in Decker and Parker (1993). (C) The identities of the additional bands in dT-treated samples are unknown but may arise from minor PGK1pG mRNA species with longer 3' ends.

Yeast transcripts are decapped without deadenylation in a conditional *pab1* mutant

The preceding result suggested that mRNAs are decapped prior to deadenylation in the absence of Pab1p. However, because the experiment was done in a strain also carrying the *spb2Δ* mutation, it was necessary to

verify that deadenylation-independent decapping was due solely to the loss of Pab1p function. To accomplish this goal we constructed a conditional *PAB1* strain by replacing the chromosomal *PAB1* gene in a wild-type strain with a temperature-sensitive degen-*PAB1* fusion construct under the control of the copper-inducible *CUP1* promoter, termed *tdpab1* (see Materials and

Table 1. Strains used in this study

Strain	Genotype
yRP840	<i>MATa, trp1-Δ1, ura3-52, leu2-3,112, his4-539, cup1::LEU2pm</i>
yRP841	<i>MATa, trp1-Δ1, ura3-52, leu2-3,112, lys2-201, cup1::LEU2pm</i>
yRP843	<i>MATa, trp1-Δ1, ura3-52, leu2-3,112, his4-539, cup1::LEU2pm, spb2::URA3</i>
yRP850	<i>MATa, trp1-Δ1, ura3-52, leu2-3,112, cup1::LEU2pm, pab1::URA3, [pAS137]</i>
yRP881	<i>MATa, trp1-Δ1, ura3-52, leu2-3,112, his4-539, cup1::LEU2pm, spb2::URA3, pab1::URA3</i>
yRP884	<i>MATa, trp1-Δ1, ura3-52, leu2-3,112, lys2-201, cup1::LEU2pm, xrn1::URA3</i>
yRP903	<i>MATa, trp1-Δ1, ura3-52, leu2-3,112, lys2-201, cup1::LEU2pm, pab1::URA3, xrn1::URA3</i>
yRP918	<i>MATa, trp1-Δ1, ura3-52, leu2-3,112, lys2-201, xrn1::URA3</i>
yRP919	<i>MATa, trp1-Δ1, ura3-52, leu2-3,112, lys2-201, ade2-101, his3Δ-200, pab1::HIS3, [pRP862]</i>
yRP920	<i>MATa, trp1-Δ1, ura3-52, leu2-3,112, lys2-201, his3Δ-200, xrn1::LEU2</i>
yRP922	<i>MATa, trp1-Δ1, ura3-52, leu2-3,112, lys2-201, pab1::tdpab1</i>
yRP923	<i>MATa, trp1-Δ1, ura3-52, leu2-3,112, lys2-201, his4-539, pab1::URA3, spb2::URA3, [pRP611]</i>
yRP927	<i>MATa, trp1-Δ1, ura3-52, leu2-3,112, lys2-201, his4-539, spb2::URA3, [pRP611]</i>

methods; Dohmen et al. 1994). In this construct the *PAB1*-coding sequences are fused to a ubiquitin-DHFR fusion protein that promotes rapid protein degradation at 36°C (Dohmen et al. 1994). Haploid *tdpab1* strains grow well at 24°C in the presence of copper but exhibit extremely slow growth at 36°C in the absence of copper (see Materials and methods). This growth defect is complemented by the presence of a wild-type *PAB1* gene (data not shown), indicating that the growth defect at high temperature is attributable to conditional *pab1* expression.

Examination of the decay products arising from the MFA2pG mRNA from *tdpab1* yeast (yRP922) grown under either permissive conditions (24°C and 0.05 mM CuSO₄), or following a 2-hr shift to restrictive conditions (36°C without CuSO₄), is shown in Figure 2. In *tdpab1* strains grown under permissive conditions most decay products from the MFA2pG mRNA had short poly(A) tails similar to those in wild-type strains, but some decay products with slightly longer poly(A) tails were also observed. Thus, the *tdpab1* allele does not fully complement the *pab1*Δ mutation under even optimal growth conditions. Following a shift to 36°C, the MFA2pG decay product from the *tdpab1* strain had a heterogeneous distribution of poly(A) tails ranging up to ~75 adenylate residues, similar to decay products in *pab1*Δ *spb2*Δ yeast (cf. Figs. 1B and 2). In contrast, decay products from wild-type cells shifted to 36°C had oligo(A)-length poly(A) tails. This observation indicates that the deadenylation-independent degradation observed in *pab1*Δ cells is at-

tributable entirely to the *pab1* lesion and is not a consequence of the *spb2*Δ mutation.

Deletion of the XRN1 gene suppresses a pab1Δ and allows for the accumulation of full-length polyadenylated decapped transcripts

The observation that decay products arising from the stable PGK1pG and unstable MFA2pG mRNAs had long poly(A) tails in *pab1*Δ *spb2*Δ and *tdpab1* strains strongly suggested that in yeast lacking Pab1p many transcripts may be decapped prematurely (i.e., without prior deadenylation). This aberrant deadenylation-independent decapping could result in premature degradation of mRNAs, which might contribute to the inviability of *pab1*Δ strains. A prediction of this hypothesis is that stabilization of mRNAs after deadenylation by blocking decapping or 5' → 3' exonucleolytic degradation would suppress the lethal phenotype of a *PAB1* deletion. To test this possibility a deletion of the *XRN1* gene, which encodes a major 5' → 3' RNA exonuclease and whose loss results in the stabilization of decapped mRNAs (Hsu and Stevens 1993; Muhlrud et al. 1994), was introduced into a diploid strain heterozygous for *pab1*Δ (see Materials and methods). Spores that carried both *xrn1*Δ and *pab1*Δ were viable and grew with a doubling time of ~6 hr, as compared to 2.5 and 10 hr for *xrn1*Δ and *pab1*Δ *spb2*Δ strains, respectively. This result indicates that a deletion of the *XRN1* gene can suppress the lethality caused by the loss of Pab1p.

The viability of the *pab1*Δ *xrn1*Δ strain allowed us to examine the nature of the deadenylation-independent degradation that occurs in a *pab1*Δ strain. For example, the presence of long poly(A) tails on MFA2pG and PGK1pG decay intermediates in *pab1*Δ *spb2*Δ and *tdpab1* strains argued that mRNAs were decapped independently from deadenylation. However, a formal alternative is that a new endonucleolytic decay pathway, which cleaved within the body of these mRNAs, had been activated in the absence of Pab1p. To distinguish between these two possibilities we tested directly whether full-length-decapped mRNAs with long poly(A) tails were produced in *pab1*Δ *xrn1*Δ yeast. This was accomplished by separating capped from decapped transcripts by immunoprecipitation of mRNAs using an antibody directed against the 5' monomethyl cap structure (Munns et al. 1982; Muhlrud et al. 1994). In this case, we examined mRNAs from a transcriptional pulse-chase (Decker and Parker 1993), in which transcription from the GAL1 promoter was rapidly induced and then inhibited. This rapid "pulse" of mRNA synthesis created a synchronous pool of mRNA whose decay was monitored in the ensuing "chase" (see Materials and methods). mRNA from a *pab1*Δ *xrn1*Δ strain was isolated at 0 and 20 min following transcription repression, and then separated into capped (pellet) and decapped (supernatant) species.

In *xrn1*Δ cells (yRP884), the MFA2pG transcripts were initially immunoprecipitable and with time accumulated as deadenylated species that were no longer immu-

Figure 2. Poly(A) tail lengths on the MFA2pG transcript and decay product from a conditional *pab1* strain. Shown are MFA2pG full-length mRNA and MFA2pG decay product from a strain harboring the conditional *tdpab1* allele (see Materials and methods) grown under permissive (24°C + 0.05 mM CuSO₄) or restrictive (36°C) conditions. Also shown are mRNA samples from a wild-type strain grown at either 24°C or 36°C. Yeast strains and growth conditions for each sample are shown at the top; poly(A) tail lengths and a schematic of the full-length mRNA and decay product are shown at left.

noprecipitable (Fig. 3A). Consistent with a requirement for deadenylation before decapping, only the deadenylated species failed to immunoprecipitate in *xrn1Δ* cells (see also Muhlrud et al. 1994). In contrast, in *pab1Δ xrn1Δ* cells (yRP903), full-length transcripts with long poly(A) tails were found in the supernatant even at the zero minute time point (Fig. 3B). Moreover, the proportion of decapped mRNA species increased at the later time point, indicating that the decapped species were the products of pre-existing capped species. The presence of full-length decapped mRNA species with poly(A) tails up to 74 nucleotides in length in *pab1Δ xrn1Δ* cells indicates that deadenylation to an oligo(A) form is not a prerequisite for decapping in the absence of Pab1p. In addition, the fact that a substantial amount (60%) of the total MFA2pG mRNA at 20 min had been decapped indicates that most, if not all, of the MFA2pG mRNA is degraded by a decapping mechanism in *pab1Δ xrn1Δ* cells, as opposed to being degraded by a new endonucleolytic pathway of mRNA decay.

An implication of *xrn1Δ* suppression of *pab1Δ* lethality is that decapped transcripts are translated to some extent. To test this prediction, we examined the distribution of capped and decapped transcripts across poly-some gradients from both *xrn1Δ* and *pab1Δ xrn1Δ* yeast,

and found that decapped transcripts were present within the gradient in positions consistent with these transcripts being loaded on ribosomes (data not shown). This result suggests that one mechanism for suppressing the lethality of *pab1Δ* is to inhibit mRNA turnover (see Discussion).

Pab1p affects the rate of, but is not required for, deadenylation

Poly(A) shortening is the first step in the decay of many yeast and mammalian transcripts (e.g., Wilson and Triesman 1988; Shyu et al. 1991; Decker and Parker 1993; Chen and Shyu 1994). Because yeast depleted of Pab1p exhibit a shift to long poly(A) tails in total cellular poly(A), it has been concluded that Pab1p is required for deadenylation (Sachs and Davis 1989). However, the degradation of mRNAs prior to deadenylation in *pab1Δ* yeast would also be expected to shift total cellular poly(A) profiles in the same manner, even if deadenylation occurs. Moreover, both MFA2pG and PGK1pG mRNAs with short and intermediate-length poly(A) tails were present in *pab1Δ spb2Δ* yeast (Figs. 1B, and 7A,B, below) suggesting that deadenylation occurs in *pab1Δ* yeast.

To determine the role of Pab1p in deadenylation we directly measured deadenylation rates of the MFA2pG and PGK1pG transcripts in both *spb2Δ* and *pab1Δ spb2Δ* cells via transcriptional pulse-chase experiments. In *spb2Δ* cells the PGK1pG mRNA at 0 min had poly(A) tails ranging in size from 44 ± 8 to 64 ± 6 nucleotides. Deadenylation of these transcripts occurred at a rate of $\sim 3.3 \pm 0.5$ nucleotides per minute to an oligo(A) form, after which the mRNA was slowly degraded (Fig. 4A). In contrast, in *pab1Δ spb2Δ* strains, the PGK1pG mRNA at 0 min had poly(A) tails 78 ± 9 to 93 ± 7 nucleotides in length. Following a temporal lag of between 10 and 20 min (Fig. 4B; see below) the PGK1pG mRNA was deadenylated at a rate of $\sim 1.2 \pm 0.5$ nucleotides per minute (as determined by measurement of poly(A) tail lengths during 5-min intervals between 20 and 60 min following transcription repression; data not shown), and was simultaneously slowly degraded (Fig. 4B). Thus, in the absence of Pab1p, the PGK1pG transcript appears to be deadenylated but at a slower rate than in a *PAB1* strain.

Experiments were also performed to determine whether deadenylation of the unstable MFA2pG mRNA was affected by the loss of Pab1p. In wild-type cells this mRNA is decapped quickly following deadenylation (Muhlrud et al. 1994; see also Decker and Parker 1993); thus, we anticipated that in the absence of Pab1p it would be decapped and degraded rapidly, concomitant with deadenylation. This rapid decay occurring simultaneously with deadenylation, could make measurement of poly(A) shortening rates difficult. To overcome this obstacle, two strategies to stabilize the full-length MFA2pG transcript were employed, with the idea that this stabilization would allow for a measurement of deadenylation without competing 5' → 3' degradation. First, transcriptional pulse-chase experiments were car-

Figure 3. Poly(A) tail distribution on capped and uncapped MFA2pG mRNA in *pab1Δ xrn1Δ* and *xrn1Δ* yeast. Total mRNA was separated into capped and uncapped fractions by immunoprecipitation with polyclonal antibodies directed against the 5' cap structure (see Materials and methods; Munns et al. 1982; Muhlrud et al. 1994), and the different fractions were resolved on acrylamide Northern gels. Shown are total (T), pellet (P), and supernatant (S) fractions from each time point; poly(A) tail lengths are depicted to the left of each panel. (A) mRNA from a transcriptional pulse-chase experiment performed in an *xrn1Δ* strain (see Materials and methods); (B) mRNA from the 0- and 20-min time points of a transcriptional pulse-chase experiment carried out in a *pab1Δ xrn1Δ* strain. The decapped mRNA species present at 0 min in B are probably derived from low levels of mRNA made prior to the transcription induction (data not shown).

Figure 4. Shortening of the poly(A) tail of the PGK1pG transcript in *spb2Δ* and *pab1Δ spb2Δ* yeast. Shown are acrylamide Northern gels of transcriptional pulse-chase experiments examining the decay of the PGK1pG mRNA in *spb2Δ* (A) and *pab1Δ spb2Δ* (B) yeast. Time points following transcription repression are shown at the top, and poly(A) tail lengths to the left, of each panel. To allow for size resolution of the poly(A) tail the 3' 319-nucleotides of the 1.4-kb PGK1pG mRNA were removed via cleavage with RNase H and oRP179 (5'-GTGACAC-CGTACTTCTTAGCG-3') prior to loading on the gel.

ried out in *pab1Δ spb2Δ* yeast in the presence of cycloheximide, which inhibits decapping but does not alter the rate of poly(A) shortening of the MFA2pG mRNA (Beelman and Parker 1994). Second, transcriptional pulse-chase experiments were performed in *pab1Δ xrn1Δ* yeast, in which full-length decapped MFA2pG transcripts are stabilized.

Transcriptional pulse-chase profiles of the MFA2pG mRNA in a *spb2Δ* strain in the absence or presence of cycloheximide are shown in Figure 5, A and B, respectively (see Materials and methods). The MFA2pG mRNA was produced with poly(A) tails of 44 ± 2 to 80 ± 0 nucleotides in length, which were shortened at a rate of 10 nucleotides per minute. This rate of deadenylation was similar to rates measured in a wild-type strain under the same conditions (data not shown) but slightly slower

than reported previously for this mRNA at 36°C (Muhlrad and Parker 1992; Decker and Parker 1993). After deadenylation to an oligo(A) form, this mRNA was degraded either rapidly (Fig. 5A) or slowly (Fig. 5B), depending on whether decapping had been inhibited by treatment with cycloheximide. In contrast, in a *pab1Δ spb2Δ* strain, the MFA2pG mRNA was produced with poly(A) tails ranging in size from 65 ± 3 to 100 ± 4 residues. Similar to the PGK1pG mRNA, little decay and deadenylation was observed for 10–20 min following transcription repression (see below). Following this lag the mRNA was simultaneously deadenylated and degraded, either rapidly (Fig. 5C) or slowly (Fig. 5D), depending on whether decapping had been inhibited by cycloheximide addition. Once deadenylation of the MFA2pG mRNA in *pab1Δ spb2Δ* yeast commenced, the rate was measured at 1.7 ± 0.4 adenylate residues per minute. Thus, deadenylation of the MFA2pG mRNA occurs in *pab1Δ* cells, although at a rate roughly sixfold slower than in *spb2Δ* yeast.

To avoid any complicating effects of cycloheximide addition, deadenylation of the MFA2pG mRNA was measured in transcriptional pulse-chase experiments performed in *pab1Δ xrn1Δ* yeast. In a *xrn1Δ* strain the MFA2pG mRNA was deadenylated at a rate of 7 ± 2 nucleotides per minute such that some of the mRNA had reached an oligoadenylated form by 6 min after transcription repression (Fig. 6A); a rate slightly slower than in wild-type and *spb2Δ* strains. In contrast, in a *pab1Δ xrn1Δ* strain, following an initial 10 min-lag (see below), the MFA2pG mRNA was deadenylated at a rate of 2 ± 1 nucleotides per minute reaching an oligoadenylated form 30 min after transcription repression (Fig. 6B). Thus, deadenylation of the MFA2pG mRNA was ~ 3.5 -fold slower in *pab1Δ xrn1Δ* yeast than in a *xrn1Δ* strain and similar to rates of deadenylation measured for this mRNA in *pab1Δ spb2Δ* yeast (Fig. 5D). This result indicates further that Pab1p is not required for the deadenylation of an unstable mRNA, although in its absence, rates of poly(A) shortening are substantially reduced.

In *pab1Δ* strains both the MFA2pG and PGK1pG transcripts were decapped without a requirement for deadenylation to an oligo(A) form (Figs. 1–3). However, follow-

Figure 5. Poly(A) tail shortening of the MFA2pG mRNA in *spb2Δ* and *pab1Δ spb2Δ* yeast. Acrylamide Northern gels of transcriptional pulse-chase experiments examining the decay of the MFA2pG mRNA carried out in *spb2Δ* (A,B) and in *pab1Δ spb2Δ* (C,D) yeast. Transcriptional pulse-chase experiments shown in B and D were carried out in the presence of cycloheximide, (CHX; see Materials and methods). Minutes following transcriptional repression for each experiment are shown directly above the corresponding panel. Poly(A) tail lengths of the mRNA are shown to the left of each panel.

ing the initial lag, these mRNAs were degraded at different rates, with the MFA2pG decaying more rapidly than the PGK1pG mRNA (cf. Figs. 4B and 5C). The rapid decay of the MFA2pG, and slow decay of the PGK1pG, transcripts with long poly(A) tails in a *pab1Δ* strain therefore reflected the relative decay rates of the oligo(A) forms of these mRNAs in cells containing Pab1p (Figs. 4A and 5A; Decker and Parker 1993). This observation suggests that Pab1p does not play a role in turnover events that occur following poly(A) tail removal (see Discussion).

Additional alterations to mRNA metabolism in *pab1Δ* strains

We observed two additional phenotypes in *pab1Δ* strains during our experiments. First, the longest poly(A) tails on both the MFA2pG and PGK1pG mRNAs were longer in *pab1Δ* than in wild-type or *spb2Δ* strains. This phenomenon was observed most easily under conditions that fully resolved poly(A) tail lengths (Fig. 7A,B; see Materials and methods). Measurement of poly(A) tail lengths for the MFA2pG transcript revealed that in a *pab1Δ spb2Δ* strain the MFA2pG mRNA had poly(A) tails of up to 104 ± 5 nucleotides as compared with lengths of 84 ± 2 nucleotides in wild-type and *spb2Δ* strains (Fig. 7A). Similarly, in *pab1Δ spb2Δ* yeast the PGK1pG mRNA had tails as long as 88 ± 5 adenylate residues, whereas the PGK1pG mRNA in wild-type and *spb2Δ* yeast had poly(A) tails of up to 59 ± 4 nucleotides (Fig. 7B). The presence of these abnormally long poly(A) tails may be indicative of a loss of poly(A) tail length control during synthesis. Alternatively, mRNAs with long poly(A) tails might result from the detection of a normally produced, but rapidly processed, intermediate in mRNA biogenesis.

The second additional phenotype observed in *pab1Δ spb2Δ* and *pab1Δ xrn1Δ* strains was a temporal lag prior

Figure 6. Poly(A) tail shortening of the MFA2pG mRNA in *xrn1Δ* and *pab1Δ xrn1Δ* yeast. Acrylamide Northern gels of transcriptional pulse-chase experiments examining the decay of the MFA2pG mRNA in *xrn1Δ* (A) and *pab1Δ xrn1Δ* (B) yeast are shown. Minutes following transcription repression are given directly above, and poly(A) tail lengths on the full-length mRNAs are shown to the left of each panel.

Figure 7. Poly(A) tail lengths on the MFA2pG and PGK1pG mRNAs in *spb2Δ* and *pab1Δ spb2Δ* yeast. Poly(A) tail lengths on the MFA2pG (A) and PGK1pG (B) transcripts from *spb2Δ*, wild-type, and *pab1Δ spb2Δ* yeast were determined following separation on acrylamide Northern gels (see Materials and methods). Poly(A) tail lengths are shown to the right of each panel.

to the deadenylation and decay of both the PGK1pG and MFA2pG transcripts (Figs. 4–6). The length of this lag was similar for both mRNAs (10–20 min) and did not correspond to the time required to shorten the poly(A) tail to an oligo(A) form. This observation suggested that in cells lacking Pab1p some phase of mRNA metabolism might be delayed.

To obtain more convincing evidence of a delay before mRNA decay in *pab1* mutant strains we examined the degradation of a *PGK1* transcript that contained an early nonsense codon, termed PGK_{N103}pG. We utilized this transcript because in wild-type cells premature termination codons trigger extremely rapid mRNA decay by promoting deadenylation-independent decapping (Muhlrad and Parker 1994). Because there is no measurable delay before decay of newly synthesized PGK_{N103}pG transcripts in wild-type strains, a lag before decay of this transcript in *pab1Δ* strains should be distinguished easily.

Transcriptional pulse-chase experiments performed in *spb2Δ* yeast (yRP927) showed that the PGK_{N103}pG mRNA was degraded immediately via deadenylation-independent decapping, as it is in wild-type cells (Fig. 8A; Muhlrad and Parker 1994). In contrast, in *pab1Δ spb2Δ* cells (yRP923) the PGK_{N103}pG mRNA persisted for 15 min without deadenylation before it was degraded rap-

Figure 8. The $PGK1_{N103pG}$ mRNA exhibits a temporal lag prior to decay in $pab1\Delta$ $spb2\Delta$ yeast. Acrylamide Northern gels of transcriptional pulse-chase experiments examining the decay of the $PGK1_{N103pG}$ mRNA in $spb2\Delta$ (A) and $pab1\Delta$ $spb2\Delta$ (B) yeast are shown. Minutes following transcription repression are given directly above, and poly(A) tail lengths on the full-length mRNAs are shown to the left, of each panel.

idly (Fig. 8B). Thus, a $PGK1$ mRNA containing an early nonsense codon showed a temporal lag prior to decay similar to the lags observed for the $PGK1pG$ and $MFA2pG$ transcripts. It should be noted that following the lag, the $PGK1_{N103pG}$ mRNA was degraded rapidly. This result is in contrast to the slow decay of the $PGK1pG$ mRNA following this lag (Fig. 4B) and indicates that the recognition of the premature termination codon occurred after the lag. We interpret these observations to suggest that Pab1p functions in additional phases of mRNA biogenesis such as cytoplasmic messenger ribonucleoprotein (mRNP) maturation. Because $pab1$ mutant strains do not accumulate poly(A)⁺ mRNA in the nucleus, it is unlikely that Pab1p is required for nuclear–cytoplasmic transport (Kadowaki et al. 1992). One simple model is that Pab1p might be required for the remodeling of mRNP structure that occurs shortly after nuclear–cytoplasmic transport and involves the exchange of hnRNP proteins for cytoplasmic mRNA-binding proteins such as Pab1p (Piñol-Roma and Dreyfuss 1992; Anderson et al. 1993; Matunis et al. 1993; Flach et al. 1994).

Discussion

Pab1p normally functions as an inhibitor of mRNA decapping

Two lines of evidence demonstrated that mRNAs can be decapped prior to poly(A) tail shortening in $pab1\Delta$ yeast. First, decay products of the $MFA2pG$ and $PGK1pG$ mRNAs, which arise from decapping and subsequent 5' → 3' exonucleolytic digestion (Decker and Parker 1993; Muhlrud et al. 1994, 1995), had long poly(A) tails in $pab1\Delta$ $spb2\Delta$ strains (Fig. 1B,C). $MFA2pG$ decay products with long poly(A) tails were also observed in a conditional $pab1$ strain grown under restrictive conditions demonstrating that deadenylation-independent decap-

ping was attributable specifically to the $pab1$ mutation (Fig. 2). Second, full-length-decapped $MFA2pG$ transcripts with long poly(A) tails accumulated in $pab1\Delta$ $xrn1\Delta$ yeast (Fig. 3B). We interpret these observations to indicate that Pab1p, in complex with the poly(A) tail normally functions as an inhibitor of decapping for both stable and unstable mRNAs in yeast. In the simplest model, the Pab1p/poly(A) complex would inhibit decapping in *cis* by as yet unidentified interactions, possibly involving additional proteins. This view is consistent with the observation that shortening of the poly(A) tail to a length of ~12 residues, which would be expected to promote dissociation of the last bound Pab1p molecule (Sachs et al. 1987), relieves the inhibition to decapping imposed by the poly(A) tail (Decker and Parker 1993; Muhlrud et al. 1994, 1995).

How might Pab1p inhibit decapping? One possibility is that efficient translation is required to prevent decapping. In this model the reduction in translation due to loss of Pab1p, rather than the loss of Pab1p itself, results in deadenylation-independent decapping. However, this possibility is unlikely, as in wild-type cells, mRNAs that are not translated because of the presence of strong secondary structures in their 5' UTRs still require deadenylation before decapping (Beelman and Parker 1994; Muhlrud et al. 1995). A more likely scenario is that Pab1p influences the state of a 5' → 3' interaction, which affects translation initiation and decapping rates (see below).

Control of decapping rates

The rate of decapping is regulated in at least two distinct ways. First, the Pab1p/poly(A) complex serves to block mRNA decapping (Figs. 1–3; Muhlrud et al. 1994). Second, following shortening of the poly(A) tail of an mRNA to an oligo(A) length, decapping occurs at different rates in a mRNA-specific fashion (Muhlrud and Parker 1992; Decker and Parker 1993; Muhlrud et al. 1994, 1995). The *trans*-acting factors that control rates of decapping following poly(A) tail removal are not known; however, our data suggest that Pab1p does not influence this rate. The critical observation is that the rates of deadenylation-independent decapping observed in $pab1\Delta$ strains for the $MFA2pG$ and $PGK1pG$ transcripts reflect the relative rates of decapping of the oligo-adenylated forms of these mRNAs in wild-type cells (fast for $MFA2pG$ and slow for $PGK1pG$, compare decay of these mRNAs following the initial lag in Figs. 4B and 5C). This observation suggests that gene products other than Pab1p determine transcript-specific rates of decapping following deadenylation.

Loss of Pab1p results in reduced rates of poly(A) shortening

Our data indicate that while Pab1p may affect the rate of deadenylation, it is not absolutely required for this process. The key observations were that (1) at steady state, in $pab1\Delta$ $spb2\Delta$ strains, some $MFA2pG$ and $PGK1pG$ mRNA species had significantly shortened poly(A) tails

(Figs. 1B and 7A,B), and (2) the MFA2pG and PGK1pG mRNAs deadenylated at rates 3.5 to 6 and 3 times slower respectively, in *pab1Δ* strains than in control strains (Figs. 4–6).

This finding extends a previous conclusion that Pab1p is required for deadenylation. This prior conclusion was based on the observation that there is an increase in the average length of poly(A) tails in total cellular poly(A) following a depletion of Pab1p (Sachs and Davis 1989). Our data suggest that four distinct alterations in mRNA metabolism in *pab1* mutants contribute to this change in poly(A) distributions. First, newly synthesized mRNAs had longer than normal poly(A) tails (Fig. 7). Second, these mRNAs with long poly(A) tails were fairly stable due to a temporal lag prior to the onset of their deadenylation and decay (Figs. 4B, 5C, and 8B). Third, the rates of deadenylation were reduced relative to wild-type cells (Figs. 4–6). Fourth, mRNAs were subject to decapping and 5' → 3' digestion prior to deadenylation (Figs. 1–3). Thus, the change in poly(A) tail distribution in *pab1* mutant strains is not solely attributable to an effect on the rates of deadenylation but is attributable to several alterations in mRNA metabolism.

What is the role of Pab1p in mRNA deadenylation? In the simplest model, the bulk of poly(A) shortening may be carried out by the Pab1p-dependent poly(A) nuclease that has been identified biochemically (Lowell et al. 1992; Sachs and Deardorff 1992). In this model the reduced rates of poly(A) shortening observed in cells lacking Pab1p could result from either a decrease in the activity of this Pab1p-dependent nuclease or the complete loss of Pab1p-dependent nuclease activity coupled with the continued activity of a second, Pab1p-independent nuclease. Interestingly, similar rates of deadenylation were measured in *pab1Δ* strains for the normally slowly deadenylated PGK1pG and rapidly deadenylated MFA2pG transcripts (Figs. 4–6). This result argues that the Pab1p-activated poly(A) nuclease is responsible for mRNA-specific differences in deadenylation rate. However, it should also be noted that it is possible that the slow rates of deadenylation observed in the absence of Pab1p could be attributable to proteins bound to an mRNA's poly(A) tail that are normally displaced by Pab1p. Such inappropriately bound proteins might serve as a barrier to the exonucleases that normally degrade an mRNA's poly(A) tail, resulting in a reduced rate of deadenylation.

The requirement of Pab1p for viability

It is now clear that Pab1p has multiple functions, including the stimulation of translation (for review, see Jackson and Standart 1990; Sachs 1990), the inhibition of decapping (Figs. 1–3), and influencing the rate of deadenylation (Figs. 4–6; Sachs and Davis 1989). Given these multiple functions, an important question is why the *PAB1* gene is essential. One possibility is that in the absence of Pab1p, translation rates are insufficient to allow viability. This hypothesis has been suggested based on the observations that *pab1* mutants show decreases

in polysome content and that mutations that decrease 60S subunit concentrations suppress the lethality of the *pab1Δ* mutation (Sachs and Davis 1989). Because this class of *pab1Δ* mutation suppressors includes the *spb2Δ* mutation, which does not affect mRNA decay by itself (Figs. 4 and 5; data not shown), it is probable that these suppressors act by altering translation in some manner to compensate for the loss of Pab1p.

However, our results suggest that the ability of Pab1p to inhibit mRNA decapping contributes to cell viability. The key result is that the *pab1Δ* can be suppressed by a deletion of the *XRN1* gene, which encodes the major 5' → 3' exonuclease known to function downstream of decapping during mRNA turnover. It should be noted that *xrn1Δ* mutants have a normal concentration of 60S subunits (data not shown) and thus are distinct from the suppressors discussed above. In addition, we have found that several other mutations that inhibit mRNA decay after deadenylation are also capable of suppressing a *pab1Δ* (C.A. Beelman and R. Parker, in prep.). Thus, there is a second class of *pab1Δ* suppressors that all stabilize mRNAs following deadenylation.

We propose that the *pab1Δ* mutation is lethal because of the combination of both a decrease in translation initiation and an increase in premature deadenylation-independent mRNA degradation. Both of these defects would decrease the amount of protein synthesis per transcript, with the combination reducing protein production below what is required for viability. This view of Pab1p function would be analogous to a case of synthetic lethality except the two distinct functions would reside within a single multifunctional protein instead of within two different gene products. Consistent with this hypothesis, no single part of Pab1p is essential for viability at 30°C (Sachs et al. 1987). This model would also explain the two distinct classes of *pab1Δ* mutation suppressors. One class suppresses by partially compensating for inefficient translation initiation, and the other suppresses by stabilizing transcripts that would otherwise be degraded prematurely. Such a model also predicts that there should be viable alleles of *PAB1* that separate the functions of translation initiation and inhibition of mRNA decapping.

Materials and methods

Yeast strains

All strains used are listed in Table 1 and were grown in standard media. Appropriate selections were used for plasmid maintenance and integration selection where required. All strains except YRP19, YRP920, YRP923, and YRP927 have GAL1 upstream activating sequence (UAS)-regulated PGK1pG and MFA2pG genes (Decker and Parker 1993) as well as the *LEU2* gene (Andreadis et al. 1982), collectively termed LEU2pm, integrated at the *CUP1* locus (L. Hatfield, C. Beelman, and R. Parker, in prep.). Strain YRP843 was generated by using a 3.6-kb fragment from pAS195 (kindly provided by Dr. A. Sachs, University of California, Berkeley) that contained the *SPB2* gene (Leer et al. 1985) with nucleotides 786–1359 replaced with the *URA3* gene (Rose and Botstein 1983), to disrupt one copy of this

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gene in a yRP840/841 diploid. Sporulation of the diploid gave rise to strain yRP843. Strain yRP850 was generated by disrupting one copy of the *PAB1* gene in the yRP840/841 diploid with a 3-kb fragment from pAS72 (kindly provided by Dr. A. Sachs) carrying the *PAB1* gene (Sachs et al. 1987) with nucleotides 885–2439 replaced with the *URA3* gene. A diploid carrying the *pab1::URA3* integration was transformed with pAS137, which contains the *PAB1* gene under control of the *GAL1* UAS (Sachs and Davis 1989) and spores were germinated on media containing 2% galactose, creating strain yRP850. Both integrations were verified by Southern blot analysis (data not shown). Strain yRP881 arose from a cross between strains yRP850 and yRP843.

Suppression of a *pab1Δ*

Two independent tests of suppression of *pab1Δ* by *xrn1Δ* were performed. First, strains yRP881 and yRP918 were crossed and the *XRN1*, *PAB1*, and *SPB2* genotypes of spores from eight complete tetrads were determined via a polymerase chain reaction (PCR)-based assay (see below) and Southern blot analysis. All resulting viable *pab1Δ* spores contained either the *spb2Δ* or *xrn1Δ* mutations, strongly suggesting that the *pab1Δ* mutation was suppressed by disruptions of either of these two loci. Second, strains yRP919 and yRP920 were crossed, and diploids were cured of pRP862, which harbors a wild-type copy of the *PAB1* gene, via selection against the *URA3* gene carried on the plasmid, through growth on media containing 5-fluoro-orotic acid. These diploids were sporulated, and *PAB1* and *XRN1* genotypes of the resulting spores were determined by monitoring growth on media lacking either leucine or histidine. In all cases (48 tetrads), viable *HIS⁺* spores were also *LEU⁺*, confirming the suppression of *pab1Δ* by *xrn1Δ*.

Construction and growth of *tdpab1* strains

Strain yRP922 was created by disrupting one of the copies of *PAB1* in the yRP840/841 diploid with *Sall*–*HindIII*-linearized pRP861 (see below). Following verification by Southern analysis (data not shown), the diploid was sporulated and spores were germinated on YEPD plates containing 0.05 mM copper sulfate, creating strain yRP922. *tdpab1* haploids exhibit slow growth on both YEPD plates and in rich medium (20 hr doubling time) at 36°C. Growth of these spores is improved under the same conditions at 24°C, although still slower than that of wild-type strains. Growth rates at both 24°C and 36°C were similar to wild-type rates when medium was supplemented with 0.05 mM CuSO_4 .

PCR assay of spore genotypes

The *PAB1*, *XRN1*, and *SPB2* genotypes of spores from eight tetrads derived from the yRP881/884 diploid were determined by subjecting 50 ng of genomic DNA from each spore to PCR using the following primers: oRP159 [5′-TGAGGGTGTAAATGGGTGA-3′, nucleotides 6040–6022 from the *XRN1* gene (bottom strand)], oRP160 [5′-ATATACTTTCTACA-ATTTTTG-3′, nucleotides 547–569 of the *SPB2* gene], oRP161 [5′-ACAAAGCAGATAGATCAATC-3′, nucleotides 445–465 from the *PAB1* gene], and oRP162 [5′-GATGCGGCCAGCAAAC-3′, nucleotides 1010–1027 of the *URA3* gene]. Primer pairs 159/162, 160/162, and 161/162 give rise to products 800, 400, and 600 nucleotides long, respectively, specifically from cells harboring disruptions of the gene corresponding to primers 159, 160, and 161 (data not shown).

Plasmid construction

pPW66R (Dohmen et al. 1994) was digested with *HindIII* and filled with Klenow enzyme. Ligation of *ClaI* linkers (Promega G2151) to the blunt ends, followed by digestion with *ClaI* and religation, generated yRP858. An ~2.6-kb fragment spanning nucleotides 862–3430 of the *PAB1* gene with 5′ *ClaI* and 3′ *Sall* restriction sites, generated via PCR with primers oRP164 and 165 [5′-CGGAATTCATCGATATGGCTGATATTACTG-3′ and 5′-CGGAATTCGTCGACTTCTG CATCTTCAC-3′, respectively], was digested with *ClaI* and *Sall* and ligated to *ClaI*–*Sall*-cut yRP858, resulting in yRP859. A .77-kb *Sall*–*KpnI* fragment containing the 5′ end of the *PAB1* gene was ligated to *KpnI*–*Sall*-digested pRS404 (Sikorski and Hieter 1989), followed by digestion with *Sall* and *SacII* and subsequent ligation to a *Sall*–*SacII* fragment from pRP859 that contained the *tdpab1* construct, generating pRP861. Additional plasmids shown in Table 1 and not described previously include pRP611, which contains the *GAL1*-regulated *PGK_{N103}P_G* gene and is similar to pRP552 (Muhlrad and Parker 1994), except that the *URA3* gene is also present on the plasmid.

Growth conditions and RNA procedures

Steady-state and transcriptional pulse-chase experiments were carried out as described previously (Muhlrad and Parker 1992; Decker and Parker 1993), except that cultures were grown at 30°C (or where noted at 24°C or 36°C) in rich media containing 2% sucrose in addition to either 2% galactose (steady-state experiments) or 2% raffinose (transcriptional pulse-chase experiments). In all cases, transcriptional shutoff was accomplished through glucose repression via resuspension of cultures in rich medium containing 2% glucose. Where mentioned, cycloheximide was added to cultures (final concentration of 10 μg/ml) simultaneously with transcriptional repression. Where described, media were supplemented with copper sulfate to a final concentration of 0.05 mM. RNA isolation was performed as described previously (Caponigro et al. 1993). In all cases, RNAs were separated on 6% polyacrylamide, 8 M urea gels for 8 hr at 300 V except for the gel shown in Figure 7, which was run at 300 V for 15 hr. RNase H reactions, transfer to Zeta-Probe membrane (Bio-Rad Laboratories), and quantification and standardization of RNA levels was accomplished as detailed previously (Decker and Parker 1993), except that oligonucleotide probes specific for the MFA2pG and PGK1pG mRNAs (oRP140, 5′-ATATTGATTAGATCAGGAATTCC-3′ and oRP141, 5′-AAT-TGATCTATCGAGGAATTCC-3′, respectively) were used to probe Northern blots where described. Immunoprecipitations were carried out as described previously (Muhlrad et al. 1994), except that 5 μg instead of 10 μg of total RNA was incubated with the anti-cap antibody.

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